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Enhanced Agroproductivity by Phosphate Solubilising Bacteria

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Abstract

The demand in agricultural field can be fulfilled by providing supplements in the form of biofertilizers and biopesticides. Biofertilizers are a consortium of microorganisms that helps for the growth of plants by providing necessary nutrients either by fixing atmospheric nitrogen or by solubilising the phosphorous present in the soil. In this present study, *Bacillus coagulans* acts as phosphate solubiliser in degrading rock phosphate by providing suitable carrier material. *Bacillus coagulans* was grown in Nutrient Medium and bioprocess conditions for maximum growth of the organism was optimized. It was found that *Bacillus coagulans* showed maximum cell concentration at pH 7.5, temperature 37°C and incubation period of 24 hrs. The culture is then mixed with suitable powder and liquid carriers. Powder carrier such as water hyacinth and groundnut shell are mixed with bio inoculum. Liquid carriers such as herbal mixture solution, archaea bacterial solution and treated waste water are mixed with culture at equal proportion. Life span of the organism along with the carrier material was scrutinized for a period of 120 days. The field response of the formulated biofertilizer was checked by applying on shallots. Among the powder carriers, water hyacinth showed highest cell count of 74×10^9 cfu/ml. Comparitively, among the liquid carriers archaea bacterial solution showed highest cell count of 77×10^9 cfu/ml. In order to determine the efficiency, morphological characters of the shallots were examined.

Keywords

Bacillus coagulans; Biofertilizers; Liquid carriers; Powder carriers; Shelf-life study

Introduction

Biotechnology is gaining more attention in vast areas most importantly in the field of agriculture. It is said that agriculture is the backbone of India and also to the people in India. Agriculture is facing destructive activities because of incorporation of chemical fertilizers. The use of chemical fertilizers prevents agriculture practicing naturally. Plants require nutrients for their optimum growth and maximum yield [1]. Chemical fertilizers cause various effects to soil during application and also to humans who consume the products from it. Mainly the soil loses all the natural nutrients it possess finally leading to infertility. Soil infertility makes the land unfit for practicing agriculture thus converting the agricultural lands to industrial areas [2]. In future there is a fear of losing lands for agriculture and agriculture may become extinct. Integrated Soil Management System can be implemented in which methods for fixation of biological nitrogen and implementation of nutrients to the soil in natural method is proposed [3].

Biofertilizer is not a new term to be introduced since the natural fertilizers has been used in olden days before chemical fertilizers were introduced. These natural fertilizers have been used without the scientific knowledge of its benefits [4]. The advent of chemical fertilizers has made a drastic change in agricultural field. Chemical fertilizers help in fast growth of plants, ripening of fruits quickly and fast flowering in plants by supplying chemically synthesised NPK (Nitrogen, Phosphorous and Potassium [5]. The ill-effects caused by these chemical fertilizers are dangerous leading to extreme stages of cancer also. On spraying, these fertilizers cause air pollution by travelling along with the air to the areas distant from application.

Biofertilizers are a universally accepted mode of supply of nutrients for plant growth and development. Microorganisms rule the whole world invisibly. They are an in-depth part of soil and influence plant growth [6]. Microbes play a major role in solubilisation of insoluble phosphorous to soluble phosphorous, fixing atmospheric nitrogen, mobilization of phosphorous and production of hormones required for plant growth [7]. Though microbes are present in soil, inoculating specific microorganism along with suitable vehicle provides additional food for plants for optimum growth [8]. Different microorganism contributes in providing numerous nutrients for growth of plants.

Microorganism belonging to genus *Rhizobium* [9], *Azospirillum, Azobacter*, etc., helps in fixing atmospheric nitrogen in the soil. Nitrogen is the major nutrient required by plants mainly for their growth, development and production of fruits and grains. Crops like rice require about 18 kg of N for every tone of effective production [10-12]. Nitrogen is effectively transferred from mature leaves immediately to the young leaves. So this mobilization of nitrogen is indicated by the deficiency symptoms in the mature leaves [13]. *Bacillus, Pseudomonas, Aspergillus*, etc., are best known for their phosphate solubilising properties. Phosphorous is second major nutrient next to nitrogen required by plants for their growth. Generally P content is low in soil. Increase in P in soil is influenced by plant development and productivity. Phosphorous supplied through chemical fertilizers is available in insoluble forms which are not easily taken by the plants. Microorganisms help in the conversion of insoluble phosphate into soluble form easily made available for plant growth [14]. Excess application of insoluble phosphorous may lead to overloading of insoluble P in soil [15]. This may lead to excessive pollution indirectly affecting runoff water [16]. Plant Growth Promoting Rhizobacteria (PGPR) is group of bacteria present on the Rhizosphere near the root surface in association with them for optimum growth and development of plants [17]. PGPR through its motility reaches actively on the surface of roots and easily adapts to the changing environment [18]. Bacterial species of *Pseudomonas* [19], *Bacillus, Klebiella, Enterobacter*, etc., are important in possessing these characteristics [20].

The present study deals with *Bacillus coagulans* plant growth promoting Rhizobacteria in acting as biocontrol agent for plant growth. Carrier material serves as vehicle for the microorganisms to provide nutrients. Carrier material must possess good moisture

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absorption property, non-toxic, inexpensive, easily available, etc. In this study carrier material is formulated in such a way that they provide additional nutrients to the plant for their growth.

Material and Methods

Procurement of organism

The microorganism was procured from Bannari Amman Bio labs in Sathyamangalam. The organism was transferred to laboratory for reviving and was refrigerated for further use. *Bacillus coagulans* was revived in Nutrient Medium providing suitable growth conditions.

Optimization of growth conditions

Conditions for optimum growth of *Bacillus coagulans* were optimized by varying pH, temperature and incubation period. Optimization was performed using simple method. The organism was grown at different pH (6,6.5,7,7.5,8), temperature (27,30,33,37,40) and incubation period (12,24,36,48,60).

Selection of carrier materials

Powder carriers: Water hyacinth and Groundnut shell were selected for this study. The collected samples were transferred to laboratory. The carriers were dried in shade for about 2-3 days to remove moisture content fully. Then the carrier materials were grinded to fine powders. After grinding, the carrier materials were sieved such that the size of the carriers ranges between 10-40 µm. The grinded carrier materials were stored in sterile containers for future use.

Liquid carriers: Domestic and agricultural wastes are chosen as liquid carriers. Three liquid carriers were formulated namely i) Herbal Mixture Solution ii) Archaea Bacterial Solution iii) Waste water. Herbal Mixture Solution formulation contains butter milk + *Terminalia chebula* (sweet wood (adhimadhuram)) + *Glycyrrhiza glabra* (kadukai). Archaea Bacterial Solution is formulation using cow dung, jaggery, *Terminalia chebula* and *Glycyrrhiza glabra* and water. Waste water was collected from Bannari Amman Institute of Technology, Sathyamangalam. These liquid carriers were stored in sterile bottles after formulation (Figure 1).

Pretreatment of carrier materials

The powder carriers were subjected to pre-treatment process before they are mixed with the organisms. To neutralize pH, the carrier materials are mixed with calcium carbonate. Sterilization of the carrier material is essential to keep high number of inoculants bacteria on carrier for long storage period. Gamma radiation is the best way of sterilizing carrier materials. In lab scale, sterilization is usually done by autoclaving. Carrier materials are packed into thin-walled polythene bags and autoclaved at 121°C for 1 hour at 15 lbs pressure.

Carrier-based inoculum preparation

After sterilization of the carrier materials, it is mixed with the inoculums in aseptic condition. The quantity of powder carrier and culture was optimized using response surface methodology. Broth was added in such a way that no excess broth remains during blending. Then the mixture is air-dried to remove moisture content. Drying was continued till the moisture content reaches below 20%. The air-dried formulation is then packed into sterile polythene bags and tightly sealed. Liquid carriers formulations were also optimized using software Design Expert 7.0.0. The response surface methodology method reduces the total number of experiments to be performed providing optimized condition. After formulating with the organisms, liquid carriers were kept for incubation for shelf-life studies.

Shelf-life analysis

After formulating both the powder and liquid carriers, it is subjected to shelf-life analysis. Shelf-life was checked regularly once in 30 days (Figure 2). The life span of microorganism in powder carriers is determined by serial dilution method followed by plating in Nutrient medium. Then the total number of cells in various dilutions were counted using hemocytometer. For liquid carriers, the shelf-life is determined by measuring the optical density at 600nm. Serial dilution followed by plating is done and finally total viable cells are calculated using cell counter.

In vivo studies

In order to determine the efficiency of the biofertilizer produced, *in vivo* studies was performed. To about 150 grams of soil taken in beaker, one-tenth of biofertilizer was added. Powder and liquid carriers were added in separate individual beakers containing soil. One beaker without biofertilizer added was taken as control. *Allium cepa* (onion) was grown in all the beakers. Then the results were interpreted.

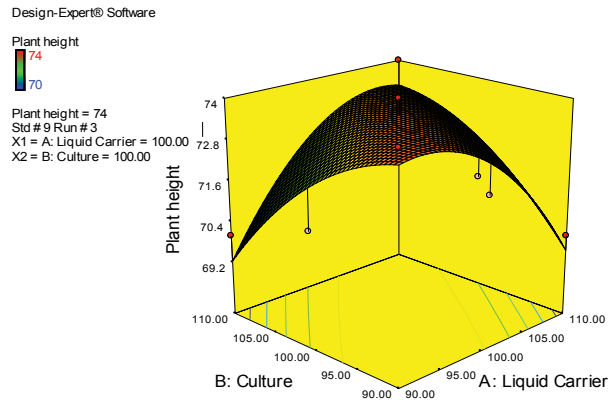


Figure 1: Optimization of liquid carrier and Bacillus coagulans culture in response surface methodology method using Design Expert 7.0.0.

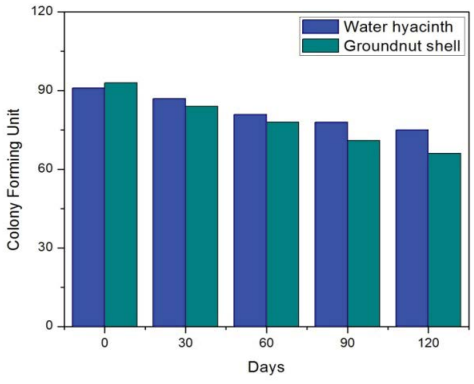


Figure 2: Graphical representation of shelf-life analysis of powder carriers.

Results and Discussion

Optimization of growth conditions

Bacillus coagulans was grown in Nutrient Medium. After optimization, it was found that *Bacillus coagulans* showed maximum growth at pH 7.5, temperature 37°C and incubation period of 24 hours (Figure 3).

Optimization of carrier and culture

The composition of blending of powder carrier and culture for maximum yield of plants were optimized using response surface methodology. On interpretation it was found that on mixing 150 g of carrier with 75 ml of broth total plant height of 73 cm is obtained. A total of 20 runs of experiments were conducted out of which ratio of 75:150 of culture and carrier resulted in production of maximum yield of plant height with increase in leaf number, leaf height, root length, bulb diameter and bulb weight.

A Normal plot of residuals was obtained which represents the yield of plant height by indication of colours. The reddish-orange colour in the (Figure 4) represents the maximum height of the plant nearly 74 cm. This indicates that the proportion of blending of powder carrier and culture was optimum to be 75 g and 150 ml respectively.

Liquid carriers

The quantity of blending of liquid carriers was also optimized using Design Expert 7.0.0 software (Figure 5). A total of 13 runs of experiments were performed. It was found that on mixing equal ratio of liquid carrier and culture, maximum yield was obtained. The total plant height was found to be 74 cm. This also resulted in increase in morphological characters of the plant such as root (root height, bulb diameter and bulb weight) and shoot (leaf number, leaf length and plant height).

The Normal Probability curve also depicts the optimised ratio of liquid carrier and culture for blending. The colour indicates the maximum growth of the plant when supplement is provided at optimized condition. The reddish-orange colour indicates the yield of plant height to be 74 cm. The 1:1 ratio of liquid carrier and culture proves to be efficient mixing for obtaining maximum yield.

Shelf-life study

The lifespan of the organism *Bacillus coagulans* was inspected for a period of 120 days. Among the powder carriers, water hyacinth showed a maximum cell count of 74×10^9 cfu/ml. The shelf-life study of *Bacillus coagulans* was analysed using Minitab 16.1.1.

Life Span in liquid carriers

The shelf-life study of *Bacillus coagulans* in liquid carriers is shown in (Figure 6). Among the three liquid carriers, Archaea bacterial solution showed maximum cell count of 77×10^9 cfu. The organism has the capacity to survive in archaea bacterial solution compared to herbal mixture solution and treated waste water.

Morphological comparison of shallots

The morphological characters such as leaf length, leaf numbers, plant height, bulb diameter, bulb weight and root length were analysed and compared among the powder and liquid carriers to determine the suitable carrier material for *Bacillus coagulans.* The shoot and root characters were analysed using Minitab 16.1.1 (Figures 7, 8, 9 and 10).

Liquid carriers

The three liquid carriers showed significant results compared to control. The morphological characters in control do not show similar results when compared with the liquid carriers. Of the three liquid carriers, archaea bacterial solution proves to be better on interpretation. The leaf length was similar and more in case of archaea

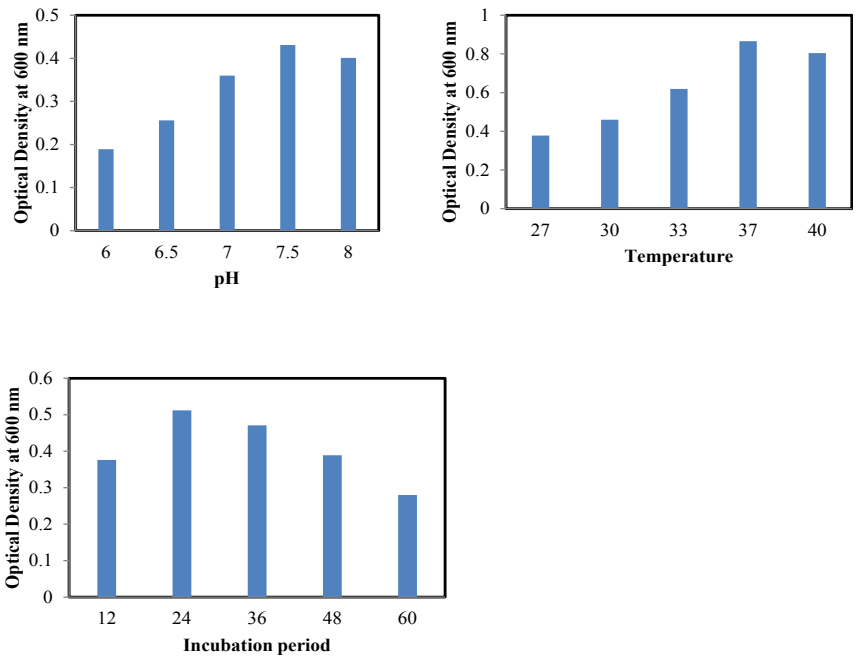


Figure 3: Optimization of growth conditions for *Bacillus coagulans*.

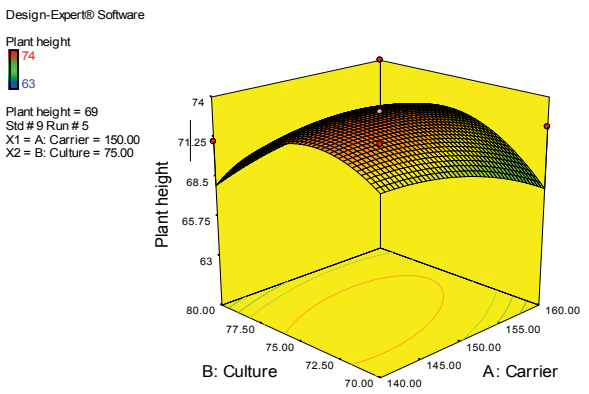


Figure 4: Optimization of quantity of powder carrier and culture of Bacillus coagulans using response surface methodology method in Design Expert – 7.0.0.

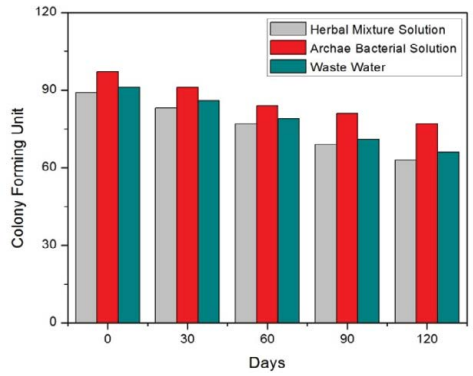


Figure 5: Graphical representation of shelf-life analysis of liquid carriers.

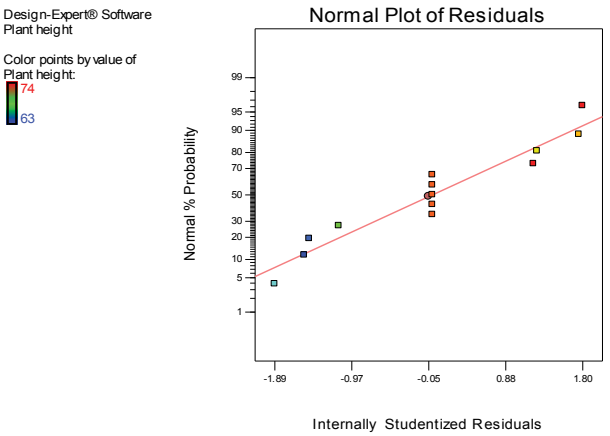
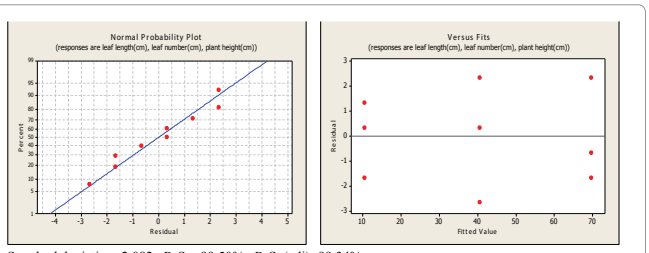


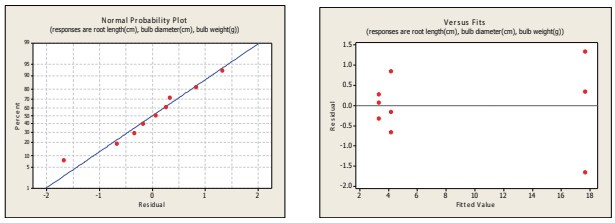
Figure 6: Normal Residual Plot of Response Surface Methodology using Design Expert 7.

bacterial solution and herbal mixture solution compared to treat waste water. The leaf number was more in archaea bacterial solution and treated waste and water. All the liquid carriers showed significant plant height but archaea bacterial solution showed increased plant height. The root length was more in treated waste water but the number of root hairs increased in archaea bacterial solution. The diameter of the



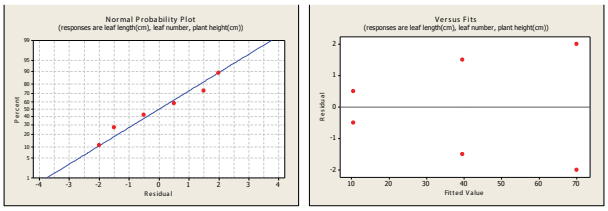
Standard deviation=2.082 R-Sq=99.50% R-Sq(adj)=99.34%
Pooled StDev=2.082

Figure 7: Analysis of shoot characters (leaf length, leaf number and plant height) using Minitab 16.1.1.



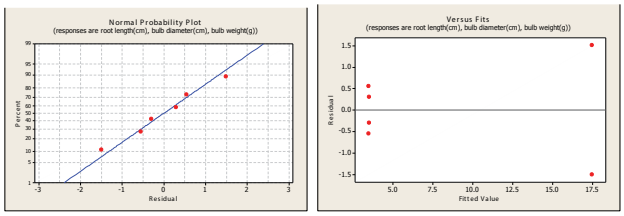
Standard deviation=1.327 R-Sq=98.02% R-Sq(adj)=96.71%
Pooled StDev=1.327

Figure 8: Analysis of root characters (root length, bulb diameter and bulb weight) using Minitab 16.1.1.



Standard deviation=2.082 R-Sq=99.63% R-Sq(adj)=99.39% ; Pooled StDev=2.082

Figure 9: Analysis of shoot characters (leaf length, leaf number and plant height) using Minitab 16.1.1.



Standard deviation=1.327 R-Sq=98.02% R-Sq(adj)=96.71%
Pooled StDev=1.327

Figure 10: Analysis of root characters (plant height, bulb diameter and bulb weight) using Minitab 16.1.1.

bulb was high in archaea bacterial solution and treated waste water. Herbal Mixture Solution showed insignificant bulb diameter. Archaea bacterial solution showed increased bulb weight compared to other carriers. Overall, Archaea bacterial solution proves to be effective and suitable carrier on scrutinising the results.

Powder carriers

Among the powder carriers, water hyacinth showed increased leaf length compared to groundnut shell. This also increased the plant height. The number of leaves for both water hyacinth and groundnut

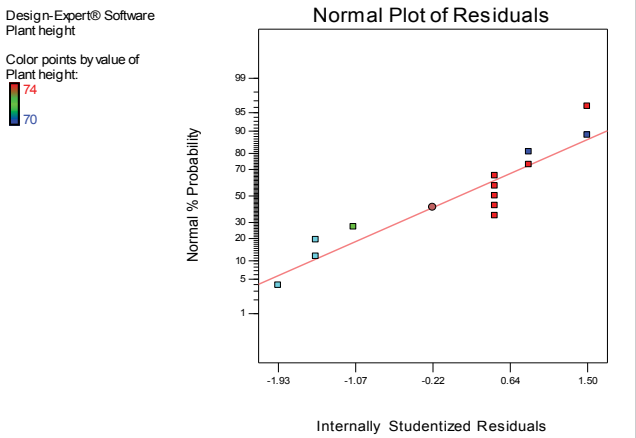


Figure 11: Normal residual plot of response surface methodology using Design Expert 7.0.0.

shell are significant. The root length was more in water hyacinth than groundnut shell. Also the number of root hairs showed high in water hyacinth only. The bulb weight was similar in case of both the powder carriers. The water hyacinth showed large bulb diameter than groundnut shell. The control showed poor results compared to both water hyacinth and groundnut shell (Figure 11).

The Normal Probability Plot for the shoot and root characters for both powder and liquid carriers depicts that the statistical data obtained from the analysis of morphological characteristics of shallots are significant. The points obtained are near and around the linear line indicating that the carriers are significant to be used as biofertilizer. Also the standard deviation obtained for powder and liquid carriers are less than 5 indicating the carrier suitable through the statistical analysis. The regression square value additionally supports the capability of the powder and liquid carrier to be best (Figure 9).

Conclusion

From this study, it was found that on culturing *Bacillus coagulans* in Nutrient Medium, the optimum conditions for effective growth were found to be at pH 7.5, temperature 37°C and incubation period of 24 hours. The highest cell concentration was found in water hyacinth with cell count of 74×10^9 cfu/ml and 77×10^9 cfu/ml in liquid carrier archaea bacterial solution. The morphological characteristics of shallots such as leaf length, leaf numbers, plant height, root length and hairs, bulb diameter and weight were found to be increased in water hyacinth powder carrier whereas in liquid carrier archaea bacterial solution leaf length, leaf number, root length and bulb diameter showed better results. Thus formulated biofertilizer with water hyacinth as powder carrier and archaea bacterial solution as liquid carrier proved to be effective than chemically synthesised fertilizers to fulfil the agricultural demand.

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